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Molecular Characterization of Guava Landraces in Kenya (Western and South Coast)

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Abstract
Guava (*Psidium guajava L*) is native to tropical areas of America where it exists as wild and cultivated. Guava has been used as source of food and in development of pharmaceuticals. Preliminarily molecular characterization has been used for the characterization of guava germplasm but molecular characterization of Kenyan guava has not been carried out. A study was carried out in 6 sites of Western and 3 sites of Coastal region of Kenya for genetic differences. Molecular characterization was done using the young apical leaves. DNA was extracted using modified CTAB method. DNA was amplified using 5 SSR markers and 3 markers produced scorable reproducable bands that ranged from 150-700bp. Levels of polymorphism ranged from 7-70% resulting in 7 cluster. 9 ISSR markers were screened and 4 produced scorable reproducible. The four primers generated bands ranging from 100-900bp. ISSR showed higher levels of polymorphism 51- 85% are and resulted in 3 major clusters. From the results of this study, molecular characterization can be used to give distinct differences among landraces
Keywords; landraces, molecular, ISSR, SSR, *Psidium guajava*

1.0 Introduction
Guava (*Psidium guajava L*) is native to tropical areas of America where it exists as wild and cultivated. In East Africa, guava grows well from sea level to an altitude of about 2,000 m above the sea level. The tree generally begins bearing 1 or 2 years after planting and continues fruiting for 30 years (Beentje, 1994). The fruit is an excellent source of vitamin C, calcium potassium and iron (Valdes *et al*., 2007). It is consumed as ripe or processed into juices; leaves are also used in traditional therapy of dysentery, diabetes, treat acute diarrhea, cough and intestinal spasmodic diseases (Ahmed *et al.,* 2011). Genetic markers have been exploited in *Martycae* family especially with guavas focusing on cultivar identification, germplasm conservation, and biodiversity evaluation and understanding genetic backgrounds of plants and animals (Ainong *et al*., 2010). The first genetic linkage map with the association of different quantitative loci already done in this crop (Valdés *et al*., 2003).Simple sequence repeats are microsatellites that consists of randomly repeated and more often identical core units containing 2-5 nucleotides and represents assign of portion of higher eukaryotic genome. SSR have been considered as markers of choice for genetic mapping, estimation of genetic resources. ISSR is a simple technique, requires no sequence information and is carried out using a single primer based on a simple repeat (Chitani, 2003). Only small amounts of DNA template are required and the results are clearly scorable and reproducible. ISSRs have been successfully used in gene tagging, variety finger printing and evaluation of microsatellite motif frequencies in the rice genome, conservation of rare plant species for many plant and animal kingdom (Sica *et al*., 2005).Very little on genetic variation of Kenyan guava and how this could be improved is unknown. There has been no formal effort in Kenya to choose suitable cultivar for the region and the market. The results of this study are useful in the discrimination of guava species and may have potential to develop the landrace.

2.0 Materials and Methods
2.1 Study site and sampling
Samples were collected from 9 site; 6 sites from Western Kenya in Butere Mumias district and three sites from Coastal region. Western sites included Kisa, Evukambuli, Bumamu, Bukura, Makunga and Sabatia. Temperatures in western ranges from 17˚C-22˚C.Coastal sites included; Shimba Ukunda and Msambweni. Coastal sites have an annual temperature of 24.2˚C. Purposive sampling technique was used and the trees under study were tagged. An average of 6 trees in each site was studied. Samples from Kisa were denoted as SHIS001-SHIS006. Evukambuli samples were labeled as EVUK001- EVUK006. Bumamu samples were labeled as BUM001-BUM006. Bukura samples were labeled as BUK001-BUK009.Samples from Makunga were labeled as MAK001-MAK006. Sabatia samples were labeled as SAB001- SAB01. Shimba samples were labeled as SHIM001-SHIM006.Ukunda samples were labeled as UK001-UK004. Msambweni samples were labeled as MSA001-MSA005.
2.2 DNA extraction
Young leaves from the tip of the plant were collected and placed in two separate 50ml falcon tubes containing silica gel. 0.4g of the leaves was weighed and ground in 3ml of extraction buffer (CTAB) as described by (Doyle

and Doyle 1990).The buffer contained (1M Tris Hcl (pH 8.0),0.5M EDTA(pH 8.0),5M NaCl2, Na2SO4, PVP10 and 2% CTAB).Leaves were crushed using mortar and pestle then incubated at 65˚C for 15mins. The samples were then centrifuged for 5mins at 13000rpm and the supernatant mixed with equal volumes of chloroform: Isoamyl alcohol (24:1). The mixture was centrifuged at 13000rpm for 5mins at the chloroform: Isoamyl alcohol step repeated and the supernatant mixed with an equal volume of cold isopropanol and incubated at room temperature. The nucleic acid was pelleted by centrifugation for 5mins at 13000rpm and the pellet washed with 70% ethanol twice. The pellet was air dried and re suspended in 50ul of sterile distilled water.0.8g of agarose was weighed in TBE buffer and heated for 2mins using microwave and ethidium bromide added. 3µl of the loading dye was mixed with 7ul of the pellet in the distilled water and loaded. Observations were made after 1hr and scored the presents of DNA bands.

2.3 PCR reaction

Five SSR primers were used for amplification of DNA (mPgCIR05, mPgCIR07, mPgCIR10, mPgCIR15 and mPgCIR16) as described by (Rodrigeuz *et al*., 2007).The primers were used to amplify 58 sample of guava collected. Each 20ul of PCR mix comprised of 10ul of 2XkapaTaq ready mix with $MgCl^{2+}$, 1ul of forward primer, 1ul of reverse primer, 1ul of DNA template and 6ul of PCR water. PCR machine was used and initial denaturation step was at 95^0C for 3mins followed by denaturation at 95^0C for 30sec, annealing was at 37-52^0C for 30sec followed with an extension at 72^0C for 2mins and with a final extension at 72^0C for 3mins that was used to terminate the reaction before a final soak at 4^0C.This was done for each primer having 40cycles. Amplified DNA was visualized on 2%agarose, 1X TBE buffer stained with ethidium bromide as described by (Manoj *et al* .,2012). Band size was estimated by visually by comparing with 100bp ladder. Bands were scored as 0 for absents and 1 for present. Genetic distance and phylogenic analysis were done using software package unweighted neighbour joining DARwins software

A total of 9 ISSR primers (UBC808, UBC810, UBC820, UBC824, UBC841, UBC843, UBC844, UBC849 and UBC 851) were used as described by (Abin *et al.,* 2011). The primers were used to amplify 58 sample of guava collected. Each 20ul of PCR mix comprised of 10ul of 2XkapaTaq ready mix with $MgCl2^{+}$, 2ul of primer, 2ul of DNA template and 6ul of PCR water. PCR machine was used and initial denaturation step was at 94˚C for 3mins followed by denaturation at 94˚C for 30sec, annealing was at 47-52˚C for 30sec followed with an extension at 72˚C for1mins and with a final extension at 72˚C for 5mins that was used to terminate the reaction before a final soak at 4˚C. This was done for each primer having 40cycles. Amplified DNA was visualized on 2%agarose, 1X TBE buffer stained with ethidium bromide as described by Xiaomei and Guochen (2012). Band size was estimated by visually by comparing with 100bp ladder. Bands were scored as 0 for absents and 1 for present. Genetic distance and phylogenic analysis were done using software package unweighted neighbour joining DARwins software

3.0 Results

3.1 Selection of polymorphic primers from candidate primers

The best and the most polymorphic SSR (mPgCIR10, mPgCIR15 and mPgCIR16) primers were selected for the study. ISSR primers selected for this study were ISSR 808, ISSR 810, ISSR 824, and ISSR 843

Table 3: Analysis of polymorphisms obtained with SSR primers in 58 guava landraces in Kenya

				Band size(bp)	
Primer	**No of amplified loci (b)**	**No. of polymorphic loci (a)**	**Polymorphism% (a/b)/100**	**Min**	**Max.**
mPgCIR10	45	7	16	150	500
mPgCIR15	37	26	70	100	700
mPgCIR16	38	3	7	300	600

The number of polymorphic bands identified in each primer ranged from 3to 26 bands (Table 3). More monomorphic bands were identified among the primers indicating low variability among the landraces, while the polymorphic band sizes ranged from 100bp to 700bp. Polymorphism analysis among the landraces revealed that the percentage polymorphism generated by the SSR primers ranged from 7% to70%

3.2 Cluster analysis using 3 SSR markers

The three SSR markers grouped the 58 landraces into 3 clusters. Cluster 1 comprised of SHIM004, SHIM006, SHIS006, MSA004, SHIS001, SHIS005, SAB003, MSA005, SHIM003, MAK004, SHIS003, EVUK005, EVUK006, SHIM002, MSA002, BUK009, EVUK004 and SHIS002. Cluster 2 comprised of BUK003, BUK001, SAB001, BUM003, SAB009, MAK002, MAK001, SAB008, SAB006, SHIM005, UKU001, UKU002, MAK003, EVUK001, EVUK002, EVUK003, SAB007, BUK002, UKUOO3, BUM005, BUM004, BUK007, BUM001, UKU004, MAK004, BUM002, MAK006 and BUM006. Cluster 3 comprised of MSA001, SHIS004,

MSA003, BUK005, SAB005, BUK004, SAB004, BUK008, SHIM001, SAB001, BUK006 and SAB002

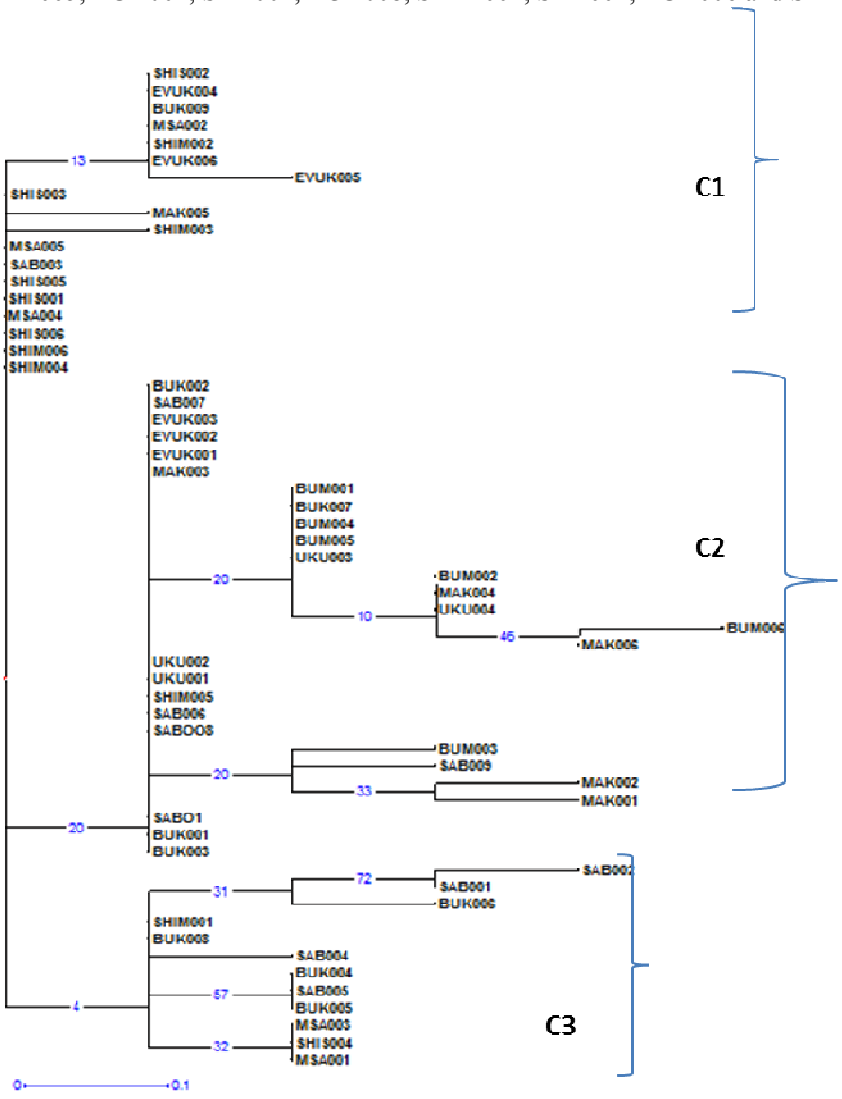

Figure 1: Cluster analysis of 58 guava landraces using 3 SSR markers

Table2: Analysis of polymorphisms obtained with ISSR primers in 58 guava landraces in Kenya

				Band size(b p)	
Primer	**No of amplified loci (b)**	**No. of polymorphic loci (a)**	**Polymorphism% (a/b)/100**	**Min**	**Max.**
UBC808	39	20	51	100	700
UBC810	50	42	84	200	900
UBC824	43	38	88	200	800
UBC843	47	40	85	300	900

The number of polymorphic bands identified in each primer ranged from 20 to 42 bands (Table 4). More monomorphic bands were identified among the primers indicating low variability among the landraces, while the polymorphic band sizes ranged from 100bp to 900bp. Polymorphism analysis among the landraces revealed that the percentage polymorphism generated by the SSR primers ranged from 51% to 88%

3.3 Cluster analysis of 58 guava landraces using 4 ISSR markers

The four ISSR markers used clustered the 58 guava landraces into 4 major clusters. Cluster 1 comprised of BUM003. Cluster 2 comprised of EVUK005, SHIS002, MSA001, SHIS001, MSA005, EVUK006, SHIM005, SHIM002, SHIS005, SHIS006, MSA002, MSA003, SAB006, MSA004, SHIM004, SHIM006, SHIS003 and

SHIS004. Cluster 3 comprised of SAB002, BUK002, BUK003, SAB005, SAB004, SAB003, SAB007, SAB01, SAB008, SAB001 and SAB009. Cluster 4 comprised of BUK005, BUK004, EVUK002, EVUK001, SHIM003, EVUK004, SHIM001, UKU004, BUK007, BUK008, BUK009, BUK006, UKU003, UKU001, UKU002, BUM002, MAK001, MAK002, BUM001, BUM005, BUM006, MAK006, MAK003, EVUK003, BUM004, MAK004 and MAK005

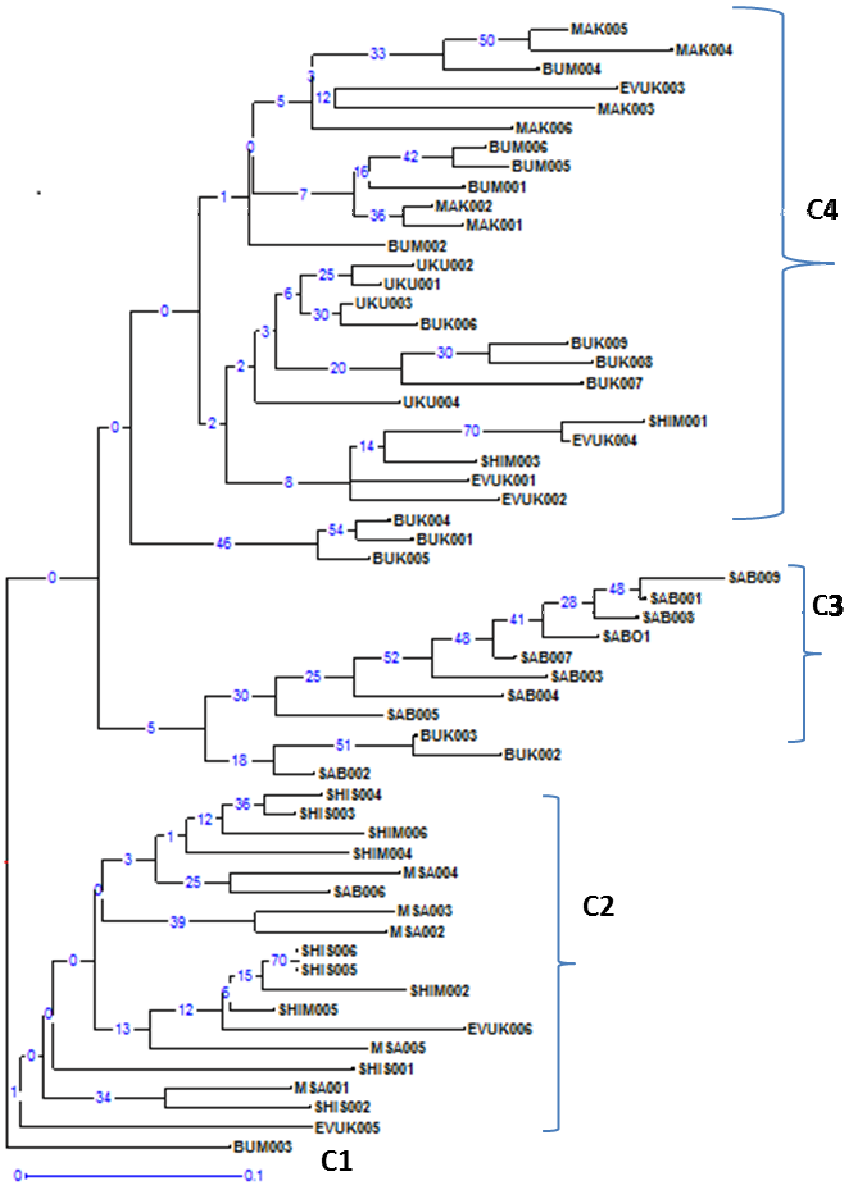

Figure 2: Cluster analysis of 58 guava landraces using 4 ISSR markers

4.0 Discussion

Genetic variability is a practical application in plant breeding for crop improvement, cultivar protection (Sozen, 2010). Several applications have been used in these studies from morphological descriptors to molecular markers. Morphological descriptors have not given clear distinction resulting to molecular markers as a good discriminant between genotypes. Molecular markers such as RPDS (Yuzbasioglu *et al*.,2006; Bibi *et al.,* 2009).AFLP (Rodriguez *et al*., 2007),SSRs (Valdes.,2007), ISSRs (Varsha and Shudhabda 2012).SSR markers have shown to be more effective in evaluating genetic diversity and phylogenetic studies (Nagaraju *et al.,* 2001; Bain *et al.,* 2011; Salima *et al* ., 2009; Kalia *et al*.,2011;Manoj *et al*., 2012).ISSRs have been used to asses genetic differences and diversity among genotypes (Schabert and Mulorstarck 2002; Sozen 2010).In this study 5 SSR and 9ISSR were used to investigate the relationship between 58 guava landraces. This is a first report on Guava landraces inn Kenya. In Various plant taxa various plants have shown to have different banding patterns with

SSR markers e.g. primer mPgCIR16 had monomorphic bands of size 300bp and 600bp .This was close to the findings of (Martin *et al*., 2004; Risterucii *et al.,* 2005; Jashi and Dhavan 2007: Laksman *et al.*,2007; Xiciomei lui and Guochen yang 2012). Primer mPgCIR16, mPgCIR15 and mPgCIR10 had polymorphic band ranging from 100-700bp. Primer mPg15 had highly polymorphic with 70% polymorphism suggesting that the primer sequence repeats are many in the guava. Four ISSR primers resulted in polymorphic banding pattern. Band size ranged from100-900bp with polymorphism of 51-85%.Among the four primers UBC843 had the highest polymorphism of 85%.The band sizes are close to the reports by (Manoj *et al.,* 2012).This high level of polymorphisms was also reported by (Abin *et al.,* 2011)

Clustering based on ISSR markers grouped the 58 landraces into 4 classes with coefficient Cluster 1 comprised of one landrace from Western region and none from Coastal. Cluster 2 had landraces from both Coastal and Western with 20.93% from western and 60% from Coastal region. Cluster 3 comprised of landraces from Western region in Sabatia and Bukura resulting to 25.58%. Cluster 4 comprised of 51.11% landraces from Western and 40% from Coastal region. Most of this landraces clustered with landraces of other geographical locations showing that despite the location the genetic make- up is still similar. Dispersal agents such as birds and humans could have led to the transfer of propagation material from one location to another (Molero *et al.,* 2003). Few landraces from similar region clustered together i.e from Sabatia and Bukura which the areas are adjacent to each other based on their geographical location indicating that because of similar growing condition the landraces appeared to be clustering together and had similar banding pattern

5.0 Conclusion

There exists genetic diversity in Coastal and Western guava landrace

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Competing interests

The authors have no competing interests

Authors' contribution

M.L.K- carried out sampling the study data scoring analysis and manuscript preparation. A. E.A-Supervised the project from sample collection analysis and correction of manuscript. A.B.N-Supervised the study and corrected the manuscript

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